Aquilos2 FIB User Guidelines ABC-061

Liu Box – Aquilos FIB

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1. **Non-Liu Lab users: User Request Email Form**

Jenny: emails & access: ID, Box-Aquilos FIB.

2. **All users: Usage Log**

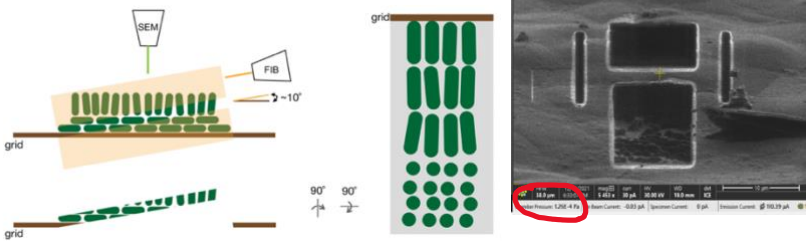
3. **Liquid Nitrogen**

- Notify Jenny **before** tank empty.
- To switch LN2 tanks:
    1. Close 'Vent' and 'Liquid' valves on empty tank.
    2. Move hoses from empty tank to new tank, connect to correct outlets (Vent - Vent, Liquid - Liquid).
    3. Open valves on new tank fully.
    4. Move empty tank to room 059C, label with paper marked 'RETURN'.

Instructions: Liu Box – Aquilos FIB – Guidelines, Access, Trainings

1. Before operation

1) Check preparation pot at **purge** state, turn on **heat** button to make sure tools dried
2) Check Chamber Pressure **< 4e-4Pa**, write exact value in Usage Log
3) Check **Beam shift** is zero
4) If nobody used Aquilos2 one day prior, perform sputtering and clean needle for 2 minutes (skip if not needed).
5) Home stage.



2. Sputtering (1st time, optional. No sample loaded at this point.)

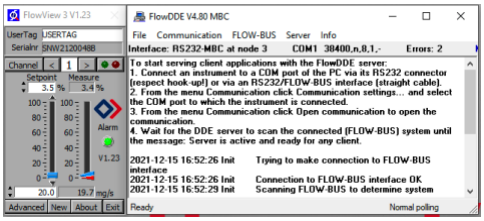
1) Prepare for sputtering (**30 mA, 10 Pa, 15 sec**)
2) Run
3) When stage stops moving, click **Recover from sputtering**.

3. Clean needle (optional)

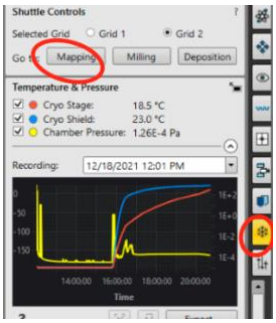
1) Change Flow Duration to 2 mins
2) **Purge**
3) For this time, no need to click Grid1 or Grid2.

4. Cool down

1) After preparation steps, start to cool down stage and shield
2) Turn on Flow DDE software
3) Communication → Open communication
4) Turn on FlowView software
5) Adjust flow rate to 200 mg/s
6) Wait until cryo-stage temperature < -190 ºC.

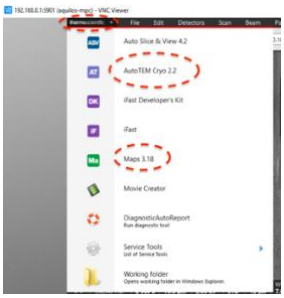


5. Load samples



1) If unsure, ask Seven for training
2) After loading sample, turn on E-beam, choose Grid1 or Grid2
3) Go to Mapping position
4) Check sample quality, such as ice, thickness, etc.
5) Decide if experiment will continue or which grid to use.
6) If good, link Z position (While E beam is on) Do this for both grid 1 and 2
7) Change detector to ETD, Mode 1: standard

6. Montage



1) Turn on software Maps 3.18
2) Create project name, choose project path…Save snapshots, Maps, AutoTEM data at: **/ShareData/User_Snapshots/*year-m-d_User_SampleName*(Collaborator)** (e.g. /ShareData/User_Snapshots/2022-01-03_Seven_Algae)
3) Change magnification on electron beam ~100x, take snapshot image on Maps. If two grids, move to other grid and take snapshot again.
4) Add tile set (choose 'seven' template):
   Set tile parameters: Tile HFW: 550 µm, Resolution: 3072*2048, Dwell time: 1 µs, Frames: 1, Focus Strategy: None, Contrast Brightness: First tile, Focus: First tile, Order: Spiral Out
5) Click Run
6) When finished, save snapshot image to local
7) Adjust eucentric positions manually (rather han AutoTem focus) to check Z-Y link, align ion beam and E beam positions when too far apart. Click ctrl + F12, then F6, left click to drag to desired position.
   Add lamella position (right side, 2nd panel of UI) after storing angle.

   After aligning all lamella positions, **uncheck** the Z-Y link.
8) To use FIB data or Cryo-CLEM images on SPC, login in browser and save to your Yale Box account
   (or temporary: Liu Box Aquilos FIB – FIB_images. Move/delete your data from this folder after FIB session).
9) Save all data (snapshots, Maps_Project, AutoTEM_Project) to:
   SharedData/User_Snapshots – Data_User_Sample (e.g. 2022-01-03_Seven_Algae). Folder saved monthly to our clusters.
   Never use USB drive on SPC (see note: No USB Allowed).
   [alternate: Seven Box Gmail: Aquilos2_User_Temp_Data: https://app.box.com/s/3jv1pxvdgbg1apmqwoxtjc5tn76db78x]

7. Import CLEM image (if needed)

1) File → Import images
2) Choose Tile Set → Alignment
3) Two options: Choose Align or Fine Alignment.

8. Choose lamellae

1) Choose good and clean areas
2) Right click, select **set lamella site here**.

9. Sputtering (2nd time, optional)

1) Prepare for sputtering (**30 mA, 10 Pa, 15 sec, No1**)
2) Run
3) When stage stops moving, click **Recover from sputtering**.

10. GIS coating

1) Select Grid1 or Grid2
2) change Flow Duration, this time depends on samples.
3) Remember this time and modify based on your milling results.

11. Sputtering (3rd time, optional)

4) Prepare for sputtering (**30 mA, 10 Pa, 15 sec**)
5) Run
6) When stage stops moving, click **Recover from sputtering**.

12. AutoTEM Cryo2.2

1) Turn on software AutoTEM Cryo2.2

2) Make sure Maps software is on, choose project "on-line", then AutoTEM and Maps link. To choose more lamellae on Maps, AutoTEM can read these simultaneously.
3) Choose template → Apply or Apply to all
4) Choose each lamella position. Click "go to" in the UI, then click "Update"
5) At this point, it is not necessary to adjust the stage position even if positions are not quite right. When all sites are finished, click "Run". Each site takes ~3 seconds.
6) Adjust the stage positons of E beam and ion beam:
   a) At UI, go to Ebeam, make sure target is centered. Image is clear (adjust focus and stigmatism, WD 7 mm helps).
   b) Adjust ion beam postion to align with E beam. When finished, adjust magnification to 10,000 X. Adjust the milling angle to a larger number (milling angle 16 degrees…change back to 8 or 9 later). Return to AutoTEM, click "Update".

7) At autoTEM, adjust rectangles to milling positions.
8) Repeat for all sites.
9) May need to click the square ("stop") in UI to manually move to next position.
10) At this point, autoTEM only suggested for rough milling, especially for cell samples, two templates:
    a) Seven bacteria fine milling
    b) Seven cell rough milling

13. Preparation (AutoTEM, not reliable)

Helps to align eucentric position:

1) Maximal tilt step: default 15°, can lower to 10° if sample too thin
2) Milling angle: default 10° (lamella surface angle) v. stage angle + 7°
3) Choose Automatic (with manual fallbacks)
4) When finished, check ion beam and electron beam to align
5) **If beam shift not huge, use shift + left drag. If beam shift huge, double click to center area of interest in E beam, then make sure √ Z-Y link is checked, use stage Z to move (ctrl+F12) to center the area of interest on ion beam also. Smaller ion beam current recommended in case sample damaged during adjustment.**
6) Default WD on ion beam 19.0 mm, electron beam 7.0 mm. Can manually change back to help adjust focus
7) When finished, manually adjust to focus well at lamella site, ideally in middle of image.
8) **If position and focus are adjusted, click "update" in AutoTEM cryo software.**
9) On AutoTEM Cryo software, adjust lamella position, then click "Continue"
10) System will move to next lamella automatically.

14. Rough milling

1) After preparation, start rough milling. Milling current is sample to sample, best to modify parameters carefully.
2) At STRESS RELIEF CUTS, Milling Current: 0.5 nA, DCM rescan interval: 120s – 300s
3) At ROUGH MILLING, Milling Current: 0.5 nA, Pattern Type: Rectangle, DCM rescan interval: 120s – 300s
4) At MEDIUM MILLING, Milling Current: 0.5-0.3 nA
5) At FINE MILLING, Milling Current: 0.5-0.3 nA
6) At FINER MILLING, Milling Current: 0.1 nA
7) Click Finer Milling-Electron Image to take snapshot after rough milling.

E beam detector change to T2, mode 3: optitilt

15. Fine milling

- After AutoTem is finished, click to go to milling position.
- **Change angle to a smaller angle (e.g. 8 or 9)**.
- Change T2 **and optitilt** (Mode 3)
- Polish lamella manually
- Suggested milling current 0.5 nA.
- Resolution (ion beam -> 3042 x 2048)
- 300 ns

- E beam (resolution 3042x 2048)
- 3 µs
- Stop at ~600 nm.
- Use 50 pA to polish lamella to < 200nm.
- Do not prolong experiments, finish before 8:00 p.m. (vacuum system not ideal).

16. Save samples

1) Make sure the sample preparation pot has cooled down 30 minutes before the last lamella thinning.

2) Take sample out of chamber
3) Turn N2 flow rate to 20 mg/s
4) Turn beam shift to zero
5) Turn off ion beam and electron beam
6) **Usage Log**: Record details in all fields and logout of Box/websites used on SPC.
7) **Grid Storage System Log**: Save milled FIB sample in puck 11 with box color, box name, sample name, session.
8) Use “sleep source” if no one using FIB 2+ days.

Do not edit below this line…

Seven 12/21:

Save FIB images to server: FIB_images/[folder with your name] / _____

Save and check lamellae in Box Sync software on FIB computer (right): Computer/Data(D:)/ShareData/BoxSync/FIB_images.

[Zoom training recordings 12/21
https://yale.box.com/s/ngtbos2sskas4ui5r5bftnyhp5nhxwss
https://yale.box.com/s/u9oqu7ao0qrr7lkf3wkhpff42lh266d1
https://yale.box.com/s/e7oiasnnl7ig7khj5btuvk3u2yespw17
https://yale.box.com/s/tocwyxjyxr37ri6lqn77jjtgcjjsmurt
https://yale.box.com/s/8l8qlcasx5nzos71vu8svuj79rnvhgx3]

Meng 11/21:

- All FIB SEM images on computer in FIB room: /desktop/shareddata/FIB images
- √ All FIB experiment records, equipment operations, expense in Box.: Liu_CryoET_Lab/Aquilos_FIB.
- Training videos in Box and: @Gunpowder: /data/disk3/FIB/FIBTrainingVideo.